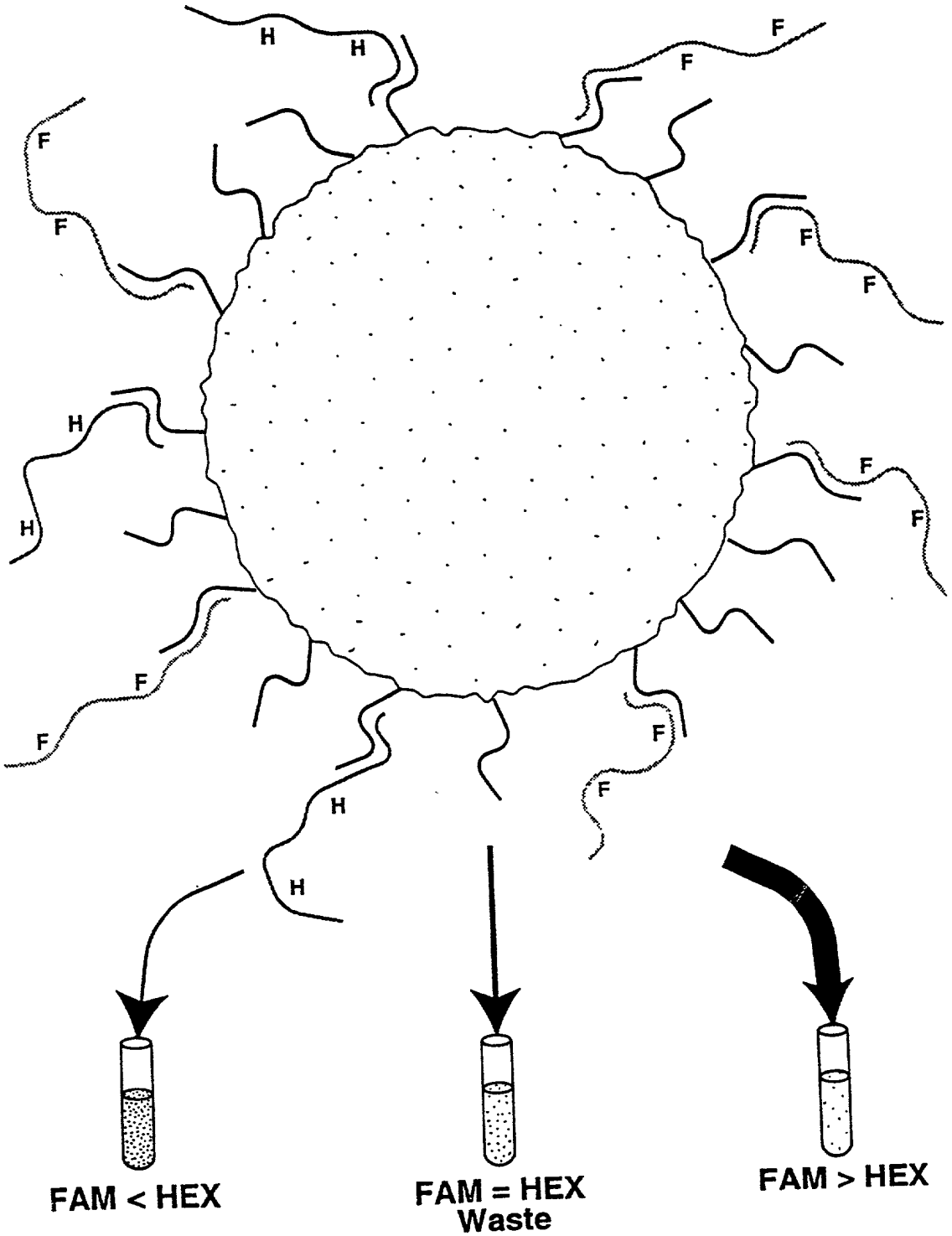


FIG. 1

Express Mail Label
No. EI125454380US

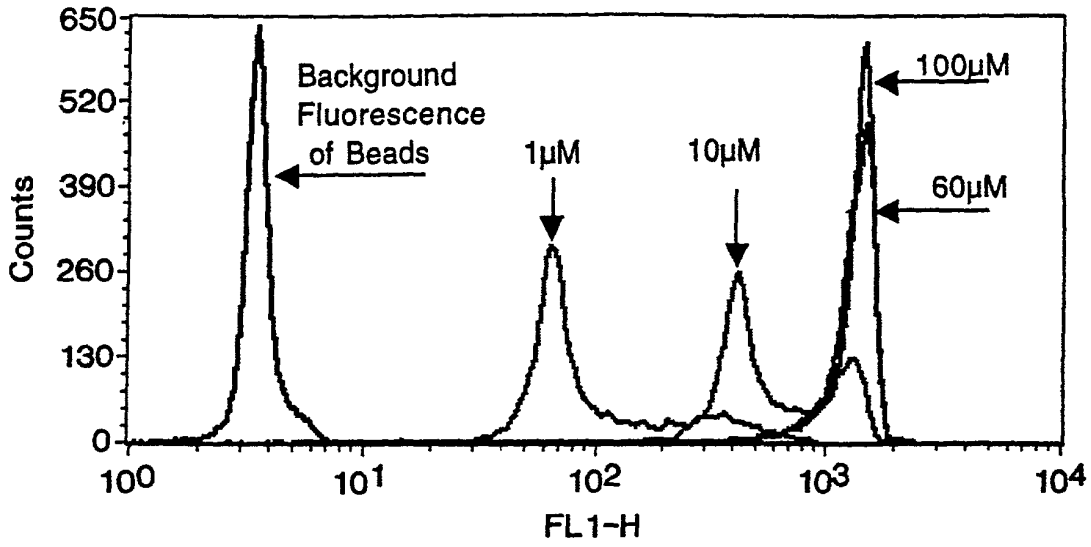


FIG. 2

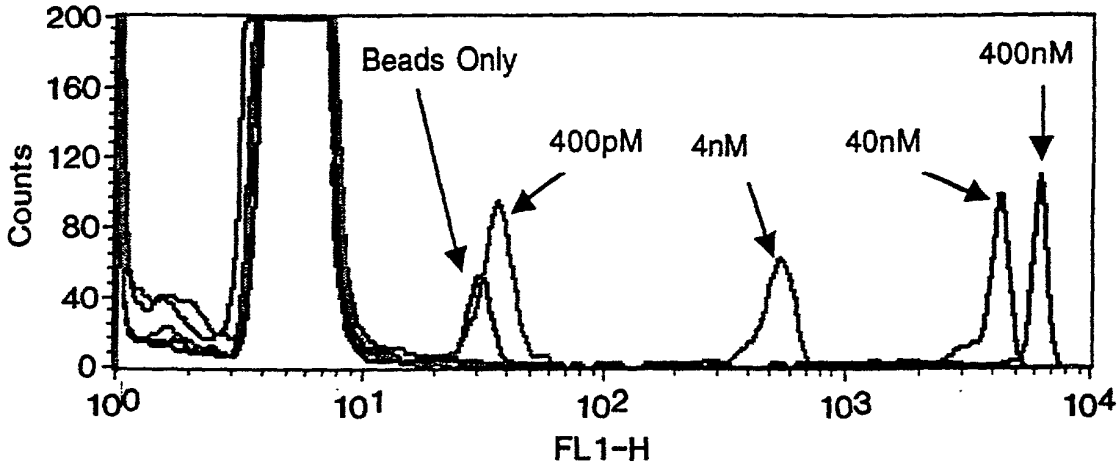


FIG. 3

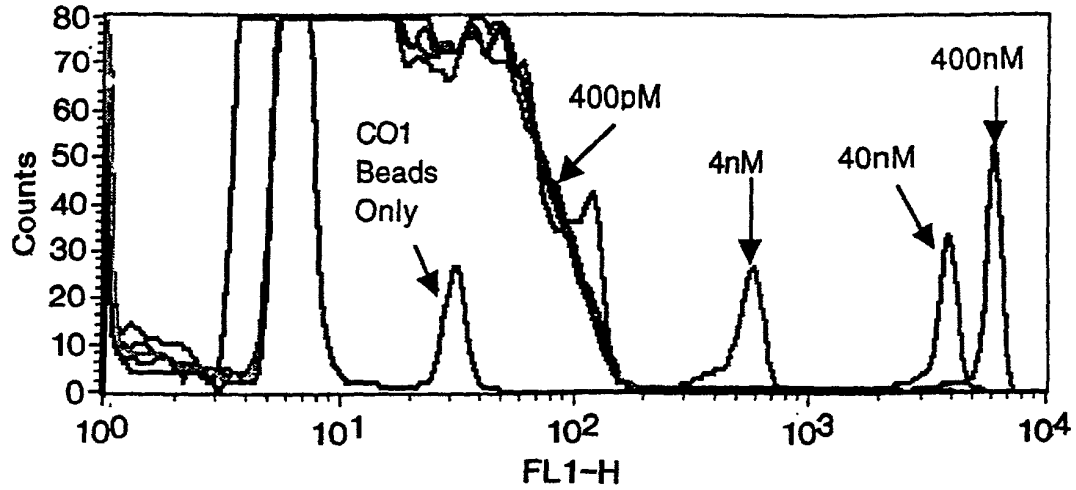


FIG. 4

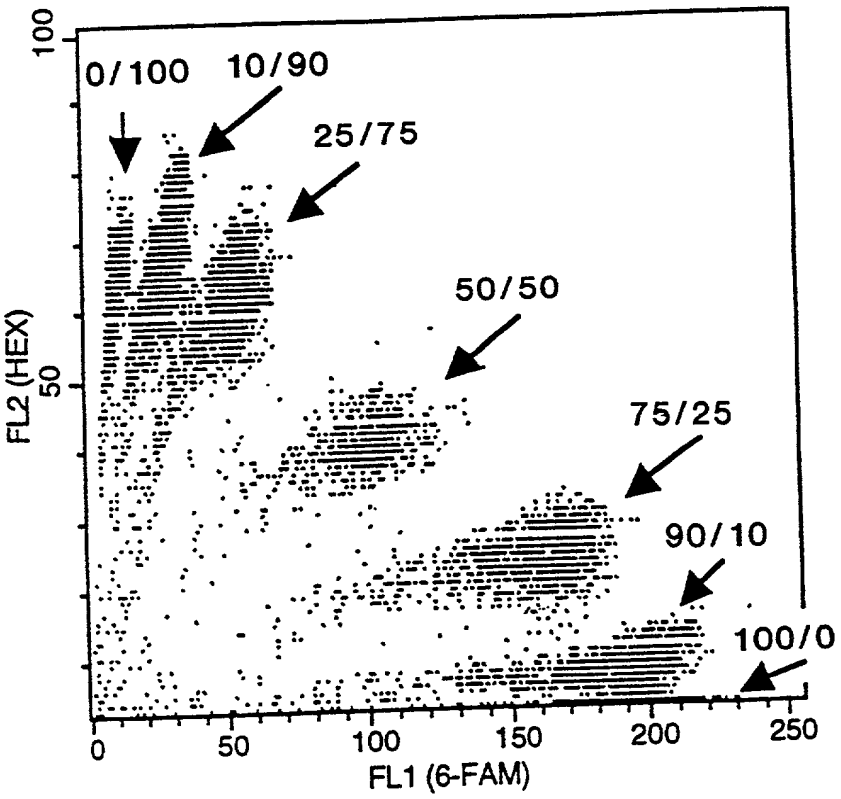


FIG. 5A

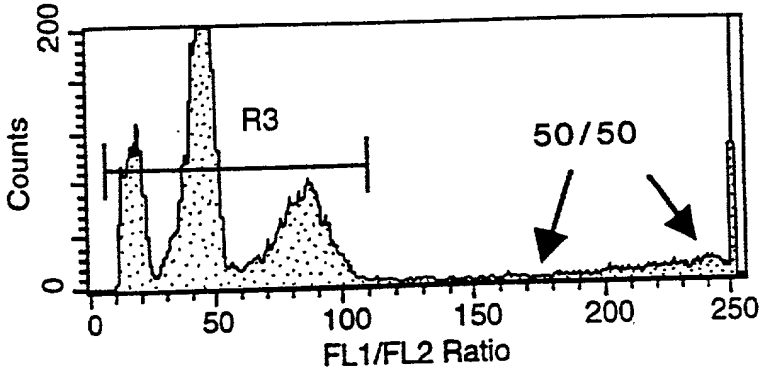


FIG. 5B-1

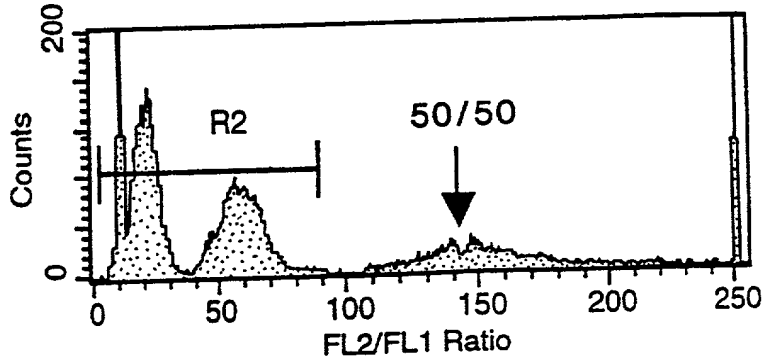


FIG. 5B-2

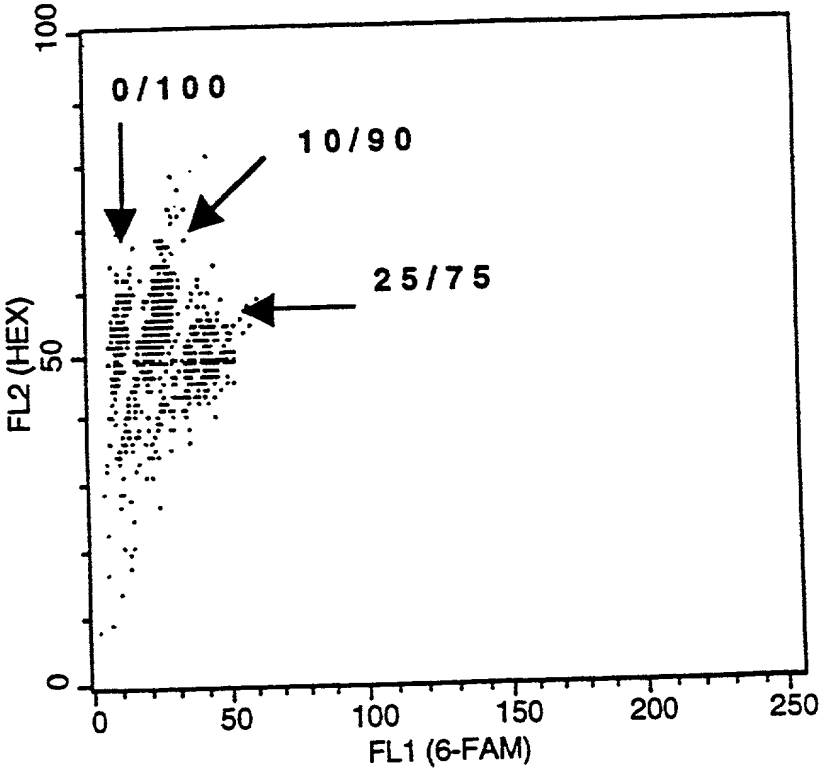

0/100
10/90
25/75
FL2 (HEX)
FL1 (6-FAM)
0
50
100
150
200
250

FIG. 5C

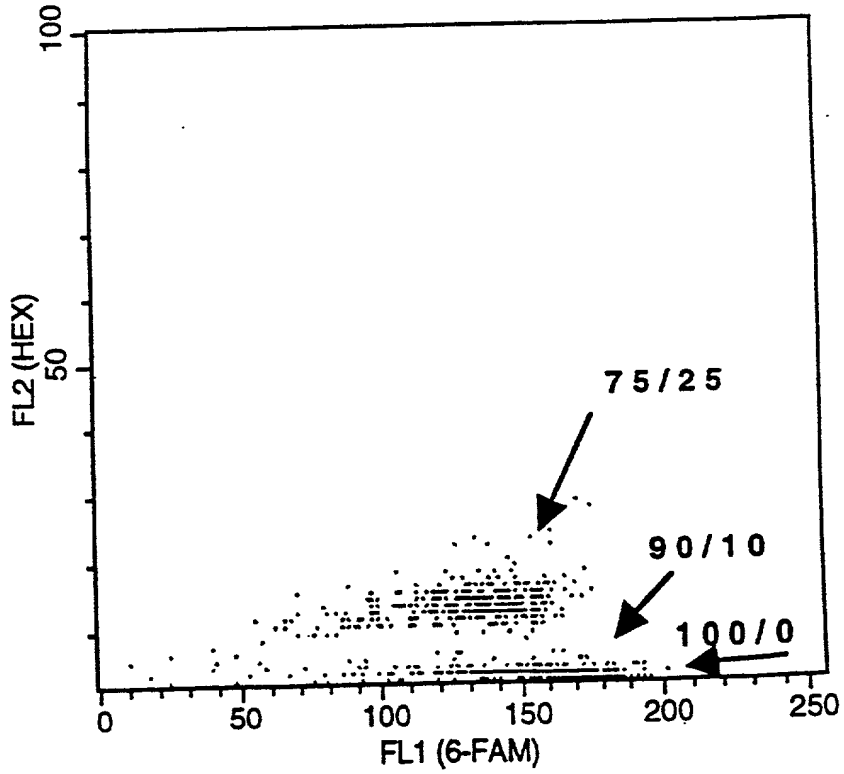

75/25
90/10
100/0
FL2 (HEX)
FL1 (6-FAM)
0
50
100
150
200
250

FIG. 5D

1. Couple to beads

2. Pool and split

3. Couple

4. Repeat....

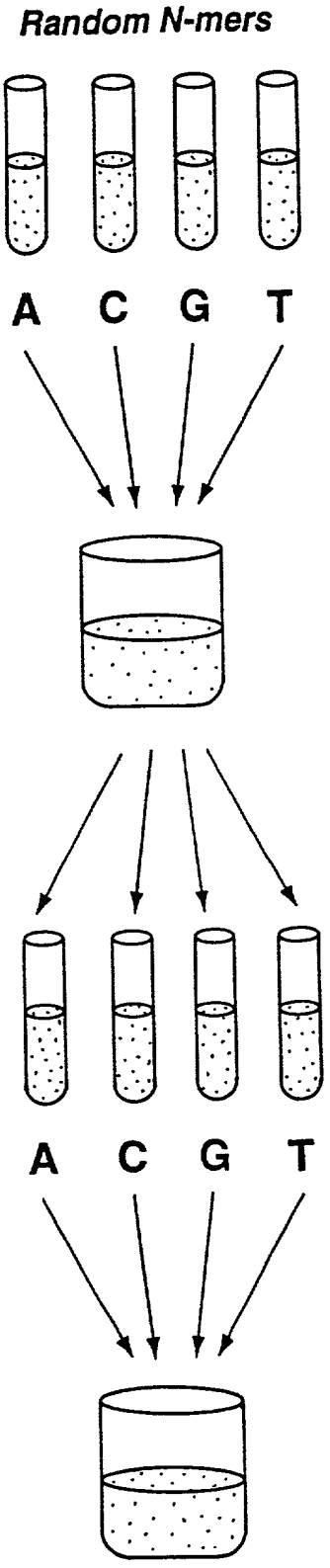


FIG. 6

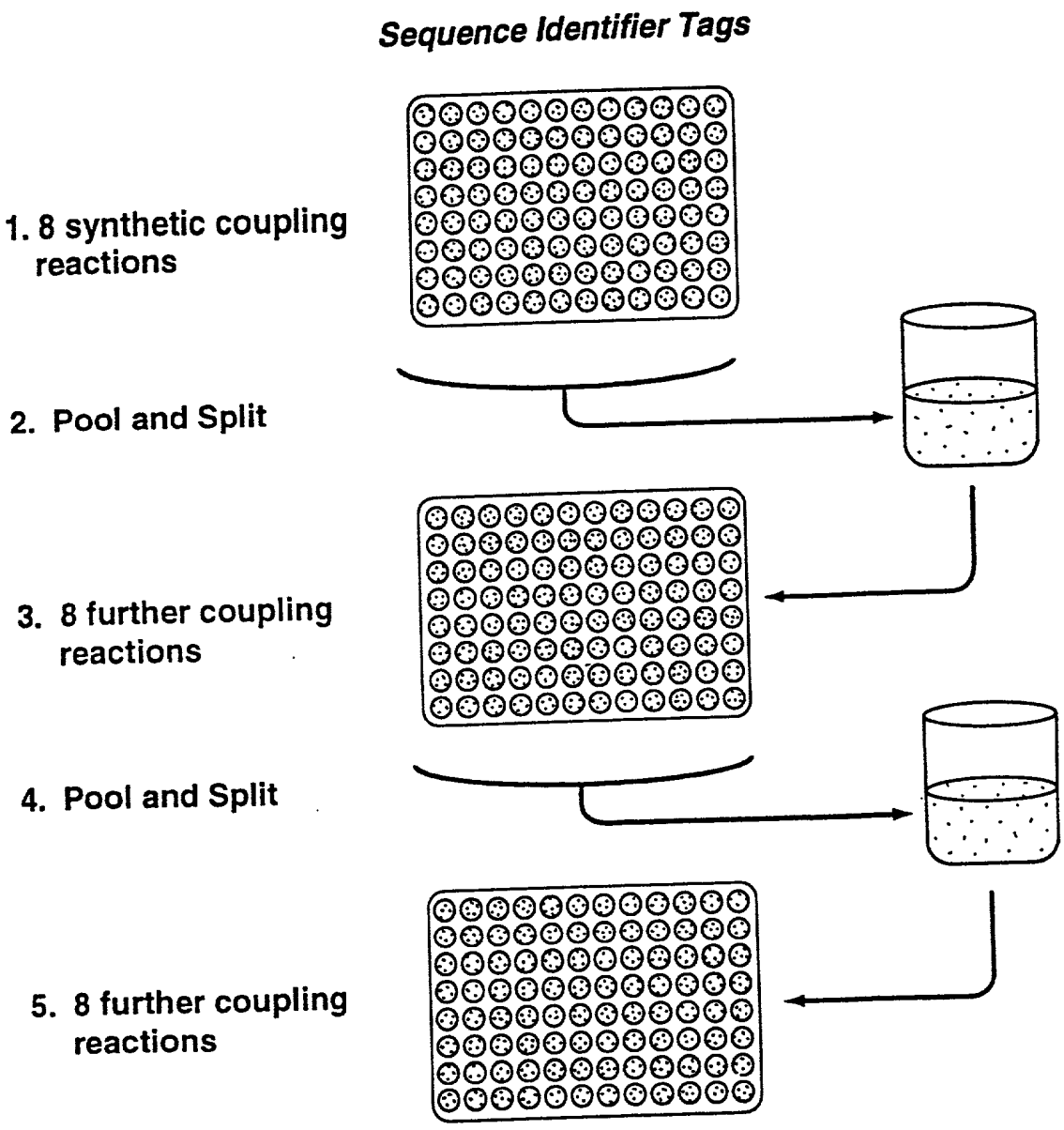


FIG. 7

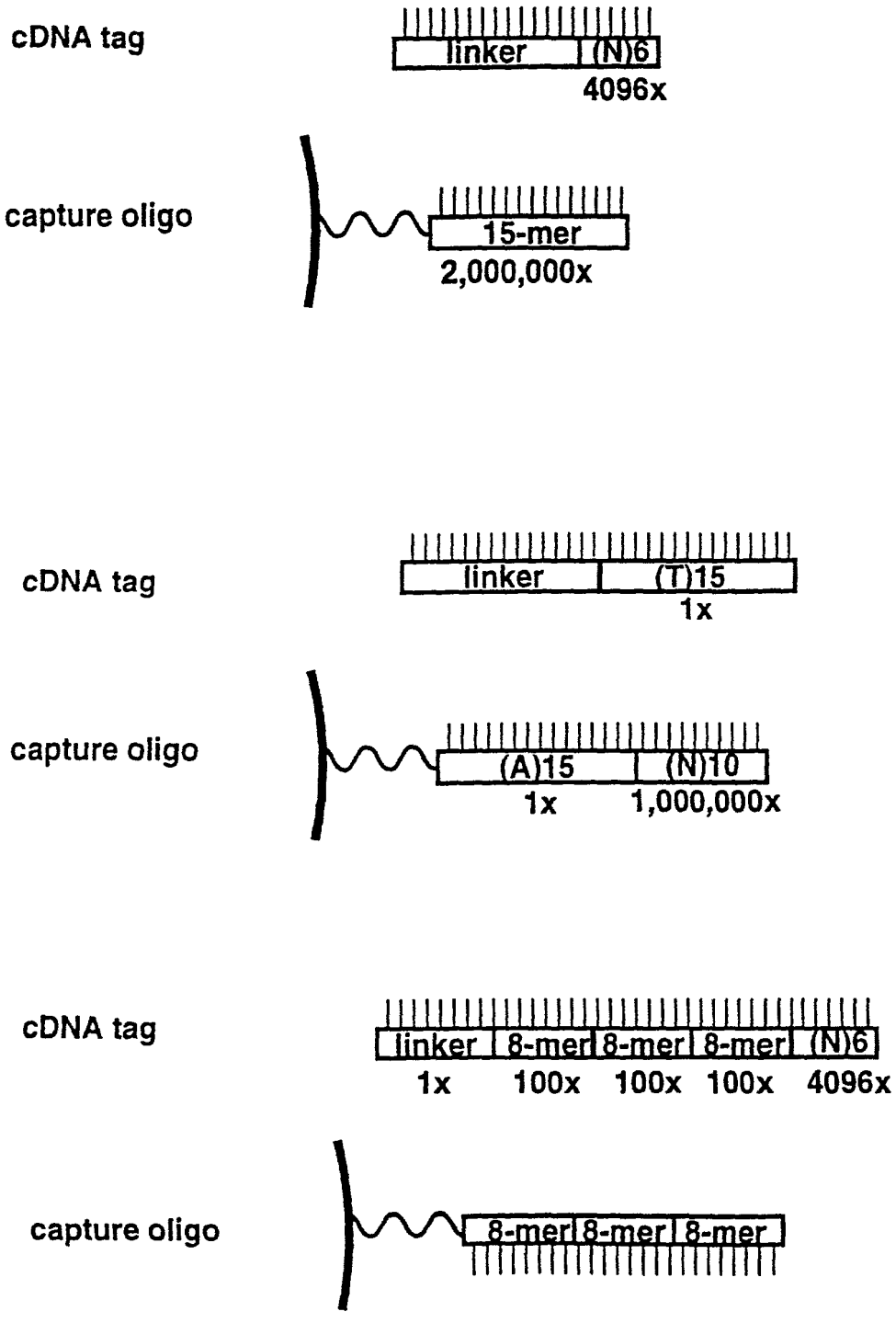
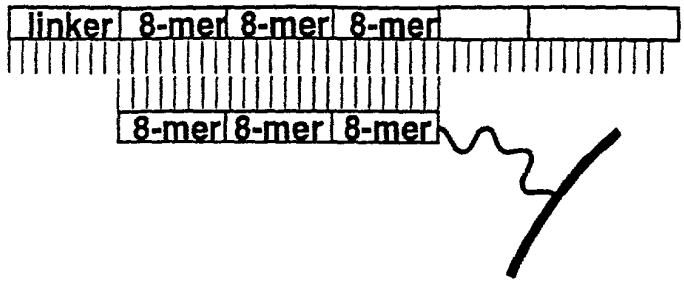


FIG. 8

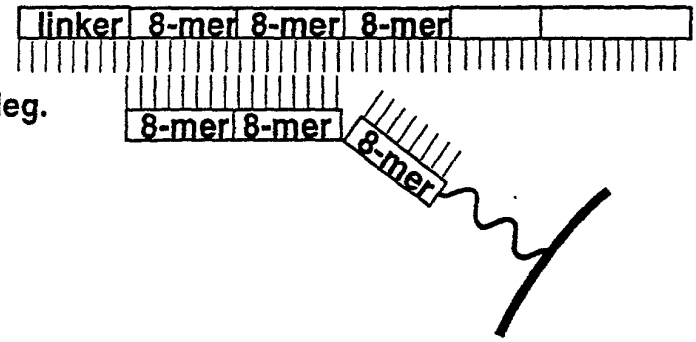
Perfect match

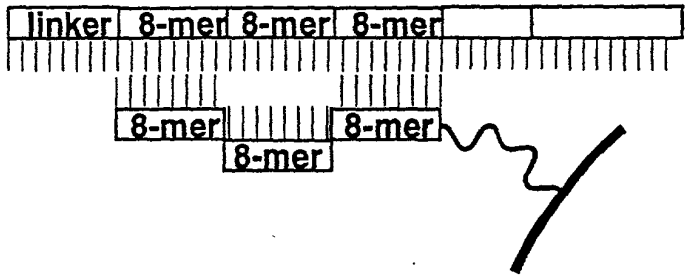
predicted Tm: 72 deg.



Mismatches

predicted Tm: <48 deg.





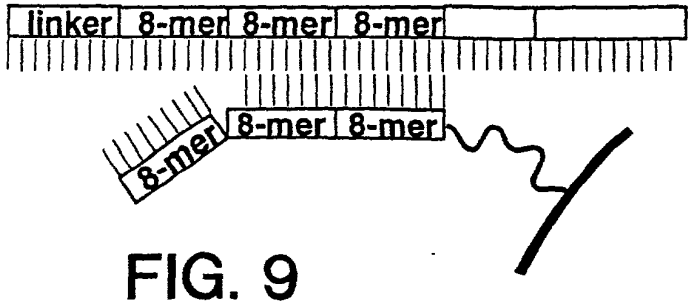
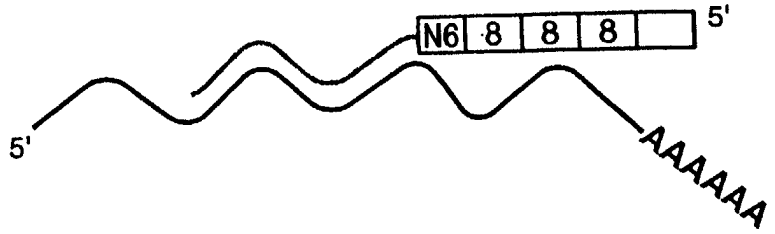
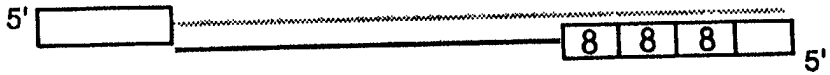


FIG. 9

1. first strand synthesis



2. second strand synthesis



3. polish ends and clone

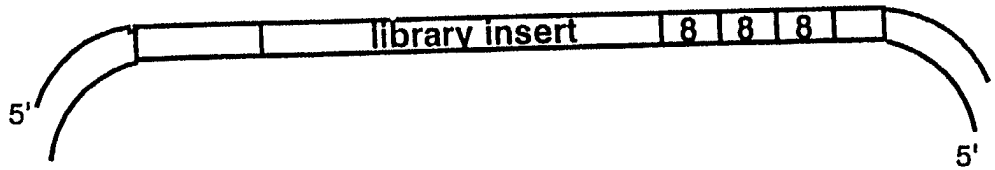


FIG. 10

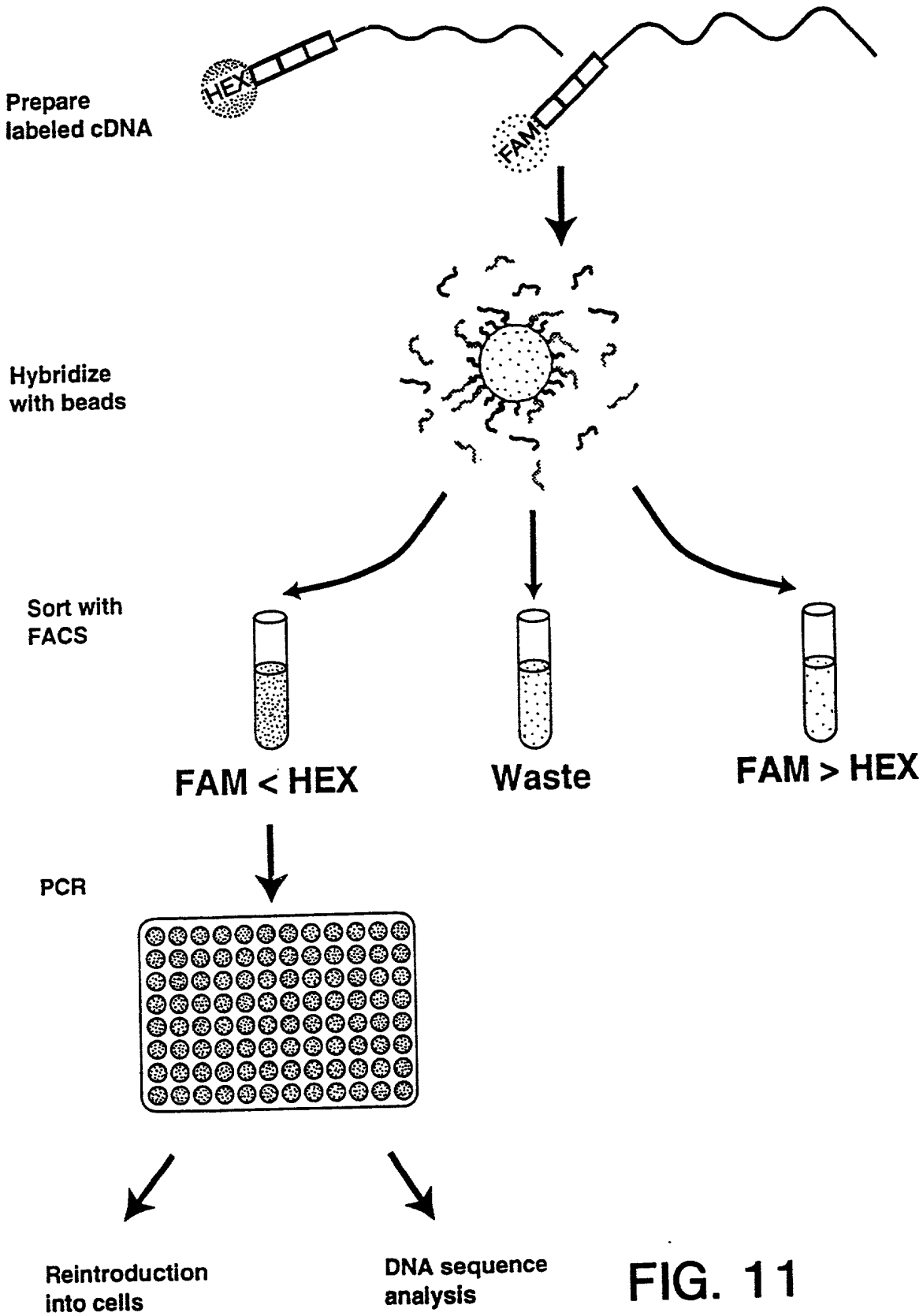

Prepare
labeled cDNA
HEX
FAM
Hybridize
with beads
Sort with
FACS
FAM < HEX
Waste
FAM > HEX
PCR
Reintroduction
into cells
DNA sequence
analysis

FIG. 11

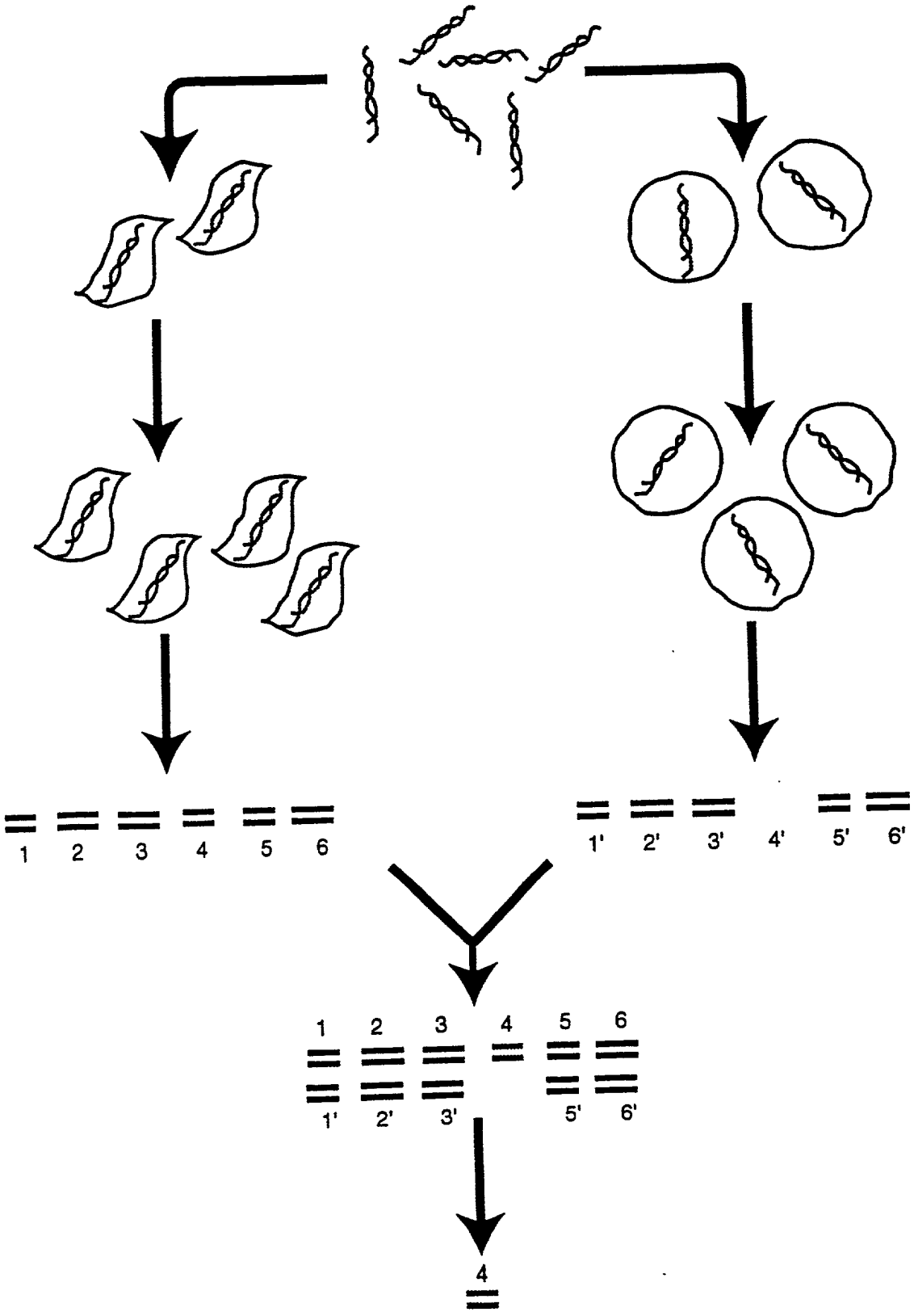


FIG. 12

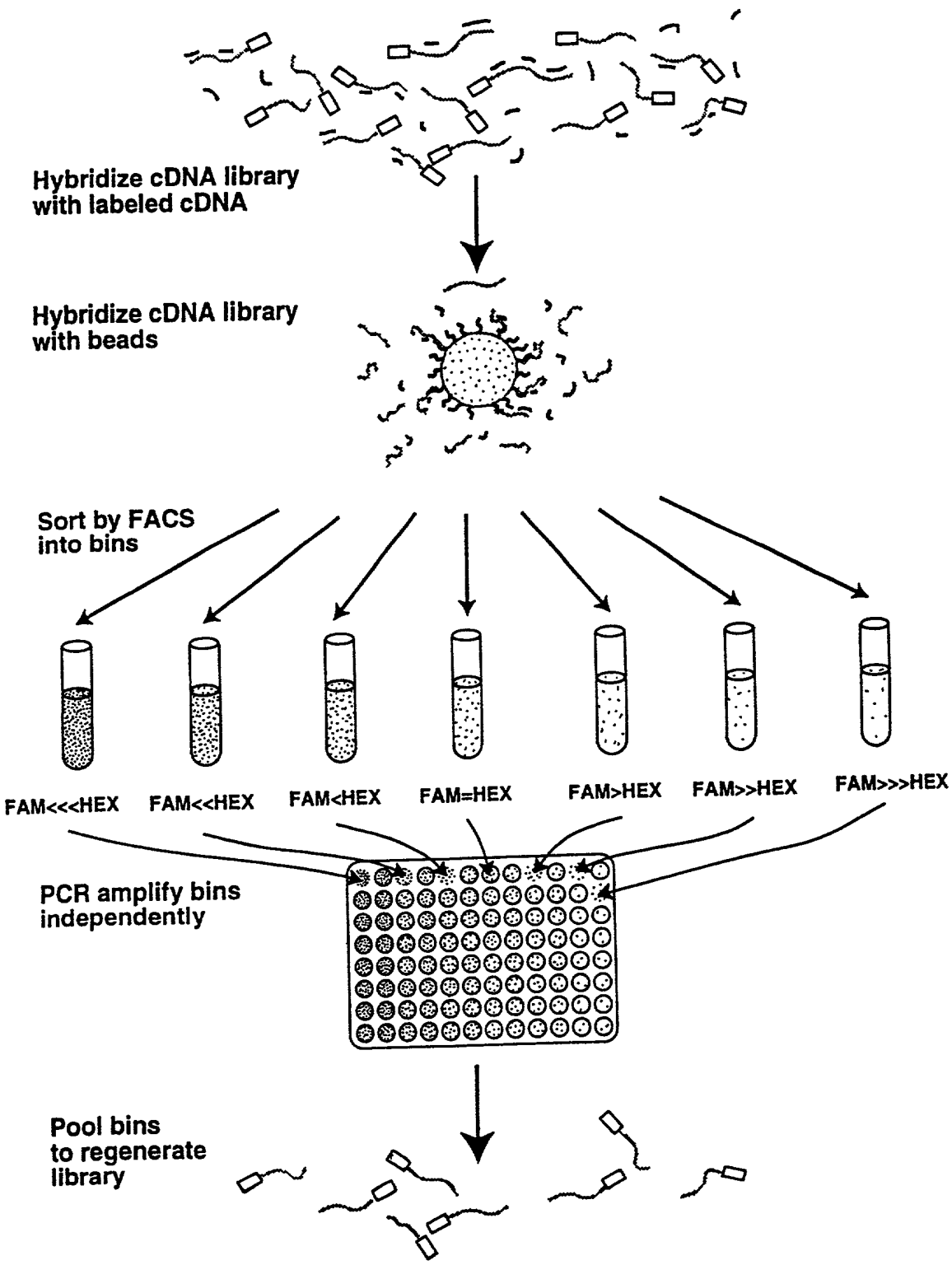

Hybridize cDNA library with labeled cDNA
Hybridize cDNA library with beads
Sort by FACS into bins
FAM<<<HEX
FAM<<HEX
FAM<HEX
FAM=HEX
FAM>HEX
FAM>>HEX
FAM>>>HEX
PCR amplify bins independently
Pool bins to regenerate library

FIG. 13

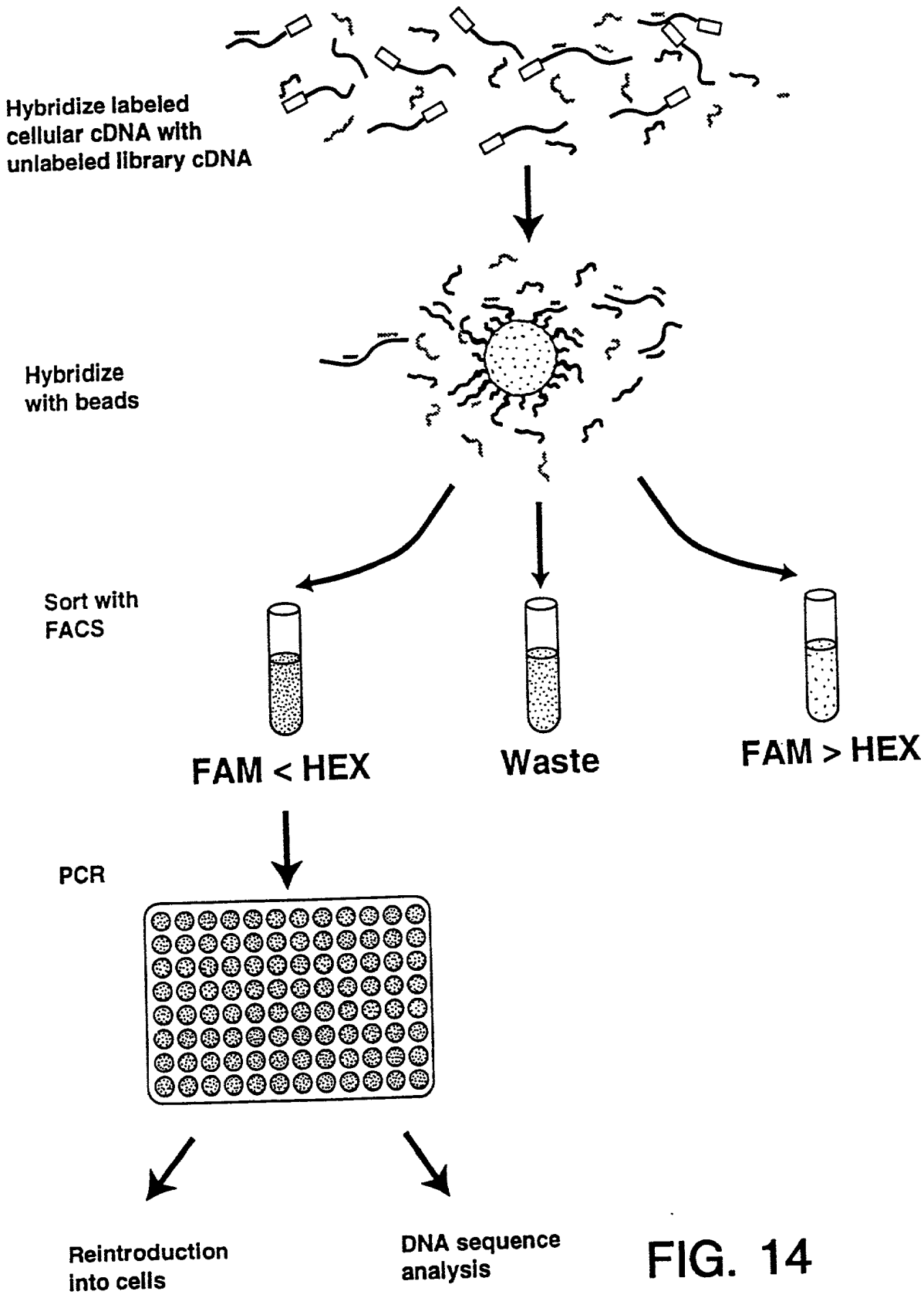


FIG. 14

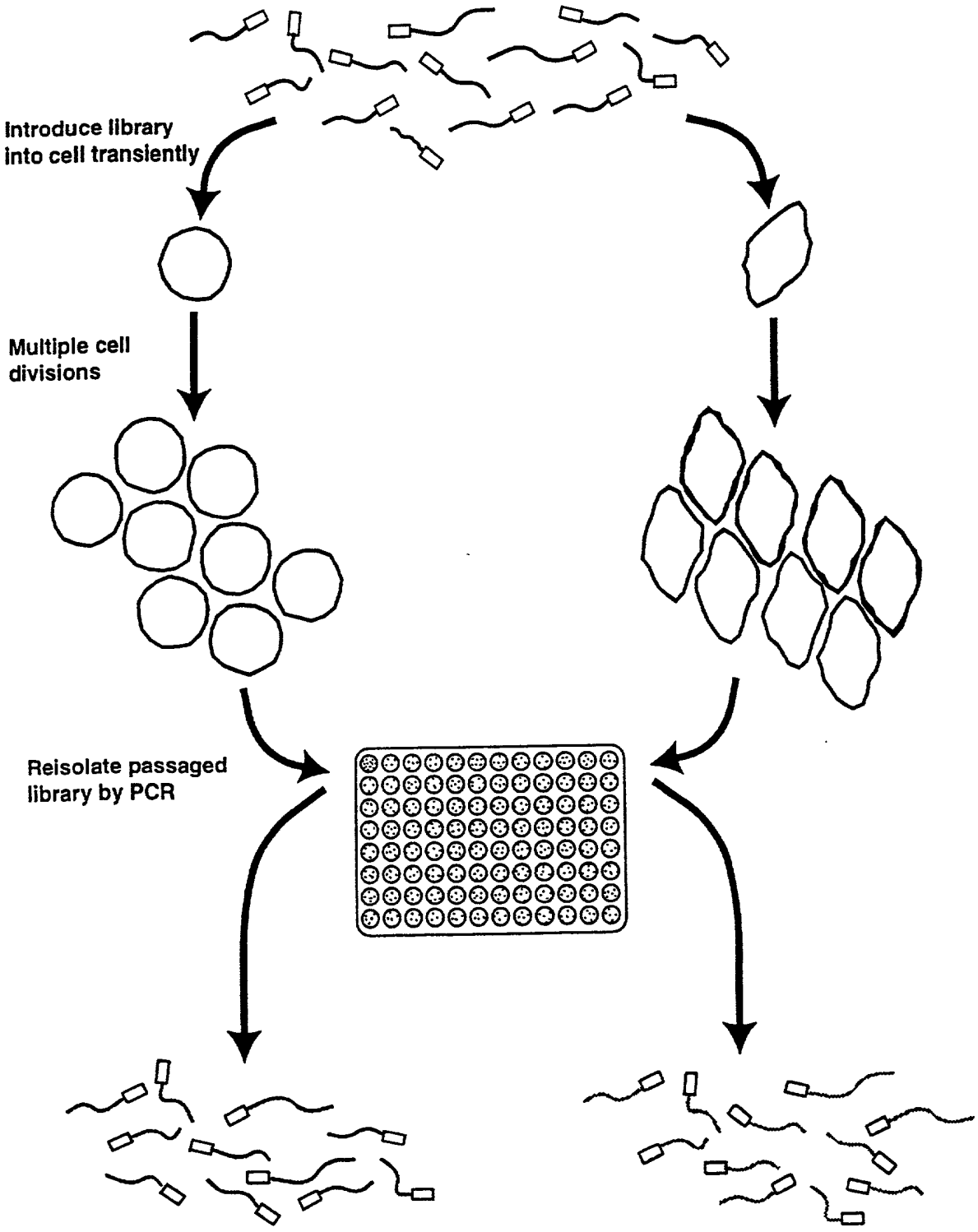

Introduce library
into cell transiently
Multiple cell
divisions
Reisolate passaged
library by PCR

FIG. 15A

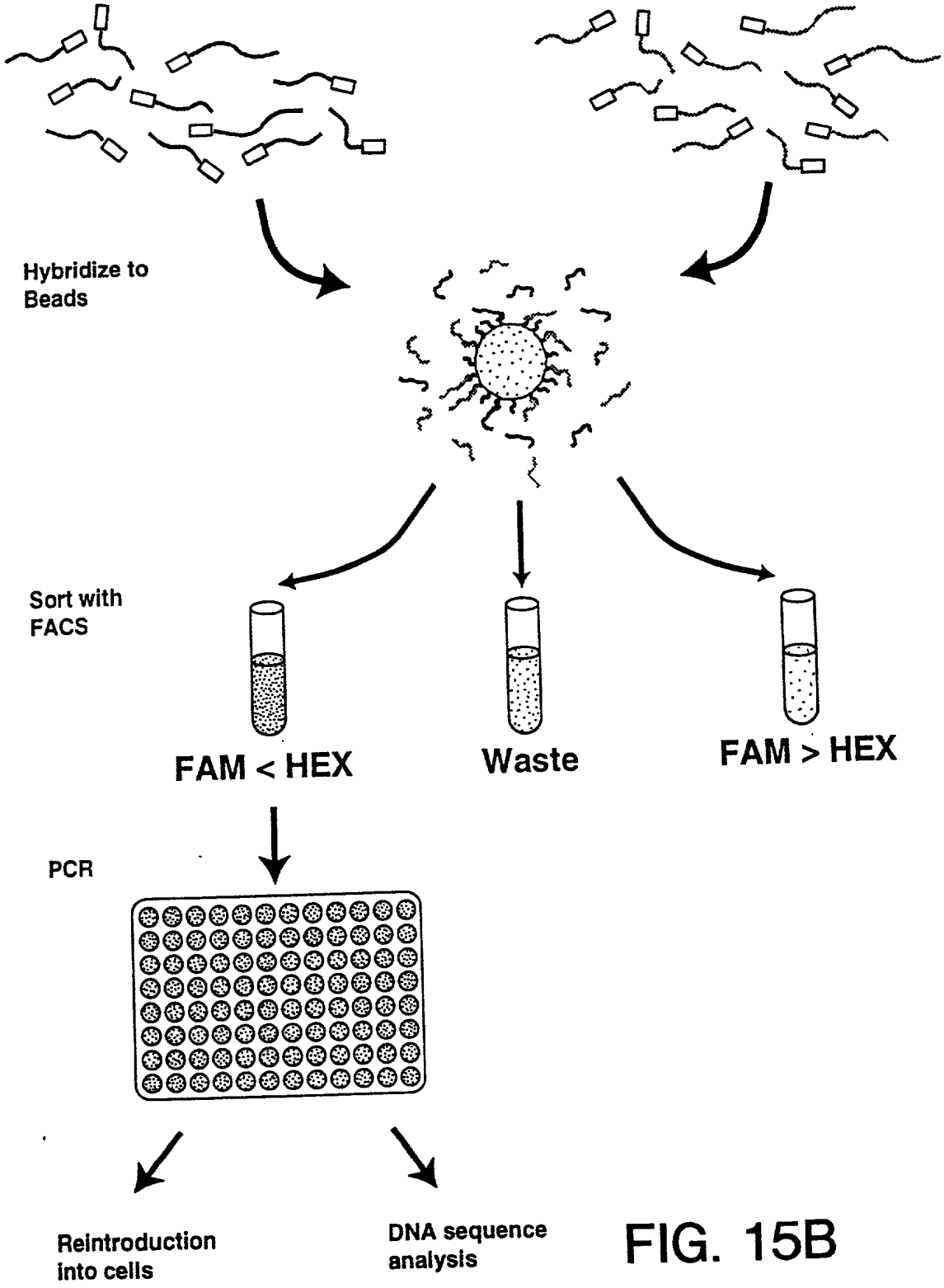


FIG. 15B

```
#include <stdio.h>

#define MATCH_NEEDED_IN_2ND 3
#define LEN_MER 8 // recompile when changed.  *things to pay attention to.

int SecondStruct(const char*);

int CrossHyb(const char *str1, const char *str2, int overlap_length);
int SimpleMatch(const char *str1, const char *str2);
char FirstN(const char *str1, const char *str2, int N);

int GC_ADDITION = 1;
int NUM_GC = 4;
int SCORE_NEEDED_IN_2ND = 7; // 1+2+4
char eq1, eq2;

FILE *fp = stdout;
FILE *fplog = stderr;

main(int argc, char **argv)
{
  int ii, jj, comp_score, s;
  int MM[LEN_MER];
  char line[256], str[LEN_MER+1];
  int gcsum, pass_gc, total_probes, failed_fn, failed_ch, failed_sm;
  char convert[4];  // 0123 to atcg convertion.
  char *compatible;

  int max_prb, cnt_prb;
  char **probe;

  int max_snd, cnt_snd;
  char **sndstr;
  int *snd_matchcnt;

  int SIMPLE_CUTOFF = 5;      // reject if this many bps match to each other,
                              // no matter where they are located.
  int CROSSHYB_CUTOFF = 9;    // 1+2+4 + 2
  int CROSSHYB_OVERLAP = 5;

  int FIRST_N = 4;

  eq1 = eq2 = ' ';
  if(argc == 1)
  {
```

FIG. 16A

```
    fprintf(stderr,
        "Usage: %s -o output_file[stdout]\n",
        argv[0]);
    fprintf(stderr,
        "\t\t-gc number_of_GCs_in_probe[%d]\n",
        NUM_GC);
    fprintf(stderr,
        "\t\t-2 secondary_structure_reject(including this value)[%d]\n",
        SCORE_NEEDED_IN_2ND);
    fprintf(stderr,
        "\t\t-ch crosshyb_reject(including this value)[%d]\n",
        CROSSHYB_CUTOFF);
    fprintf(stderr,
        "\t\t-sm simple_match_reject(including this value)[%d]\n",
        SIMPLE_CUTOFF);
    fprintf(stderr,
        "\t\t-ol crosshyb_overlap_length[%d]\n",
        CROSSHYB_OVERLAP);
    fprintf(stderr,
        "\t\t-eq add'l_equiv_bp_in_compat_checking[%c%c] (e.g., -eq gt)\n",
        eq1, eq2);
    fprintf(stderr, "\t\t-fn first_N_length[%d]\n", FIRST_N);
    fprintf(stderr, "\t\t-gc_add GC_add'l_penalty[%d]\n", GC_ADDITION);

    exit(0);
}

// parse input parameters.
ii = 1;
while(ii < argc)
 {
  if(strcmp(argv[ii], "-gc") == 0)
   sscanf(argv[ii+1], "%d", &NUM_GC);

  else if(strcmp(argv[ii], "-2") == 0)
   sscanf(argv[ii+1], "%d", &SCORE_NEEDED_IN_2ND);

  else if(strcmp(argv[ii], "-ch") == 0)
   sscanf(argv[ii+1], "%d", &CROSSHYB_CUTOFF);

  else if(strcmp(argv[ii], "-ol") == 0)
   sscanf(argv[ii+1], "%d", &CROSSHYB_OVERLAP);

  else if(strcmp(argv[ii], "-eq") == 0)
  {
```

FIG. 16B

```
  if(strlen(argv[ii+1]) == 2 ||
    (strlen(argv[ii+1]) == 3 && argv[ii+1][2] == '\n'))
  {
     eq1 = argv[ii+1][0];
     eq2 = argv[ii+1][1];
  }
  else
  {
     fprintf(stderr, "\nERROR: Invalid string after -eq flag.\n\n");
     exit(1);
  }
}

else if(strcmp(argv[ii], "-o") == 0)
 {
  if((fp = fopen(argv[ii+1], "w")) == NULL)
    {
     fprintf(stderr, "Can't open file %s to write.\n", argv[ii+1]);
     exit(1);
    }

   char logname[128];
   sprintf(logname, "%s.log", argv[ii+1]);
   if((fplog = fopen(logname, "w")) == NULL)
   {
      fprintf(stderr, "failed creating log. stderr used.\n");
      fplog = stderr;
   }
  }

  else if(strcmp(argv[ii], "-fn") == 0)
   {
      sscanf(argv[ii+1], "%d", &FIRST_N);
   }

  else if(strcmp(argv[ii], "-sm") == 0)
   {
      sscanf(argv[ii+1], "%d", &SIMPLE_CUTOFF);
   }

  else if(strcmp(argv[ii], "-gc_add") == 0)
   {
      sscanf(argv[ii+1], "%d", &GC_ADDITION);
   }
```

FIG. 16C

```
    else
     {
      fprintf(stderr, "Unknow flag %s\n", argv[ii]);
      exit(1);
     }

    ii += 2;
   }

max_prb = 30000;
probe = new char* [max_prb];
for(ii = 0; ii < max_prb; ii++)
  probe[ii] = new char [LEN_MER+1];
cnt_prb = 0;

max_snd = 5000;
sndstr = new char* [max_snd];
for(ii = 0; ii < max_snd; ii++)
  sndstr[ii] = new char [LEN_MER+1];
snd_matchcnt = new int [max_snd];
cnt_snd = 0;

// build an array of probes.  Each probe is of length LEN_MER,
// of which 'NUM_GC' are Gs or Cs.

convert[0] = 'a';
convert[1] = 't';
convert[2] = 'c';
convert[3] = 'g';

total_probes = 0;
pass_gc = 0;  // number of probes pass GC test.
for(MM[0] = 0; MM[0] < 4; MM[0]++)
for(MM[1] = 0; MM[1] < 4; MM[1]++)
for(MM[2] = 0; MM[2] < 4; MM[2]++)
for(MM[3] = 0; MM[3] < 4; MM[3]++)
for(MM[4] = 0; MM[4] < 4; MM[4]++)      //*things to pay attention to.
for(MM[5] = 0; MM[5] < 4; MM[5]++)      //*things to pay attention to.
for(MM[6] = 0; MM[6] < 4; MM[6]++)      //*things to pay attention to.
for(MM[7] = 0; MM[7] < 4; MM[7]++)      //*things to pay attention to.
{
  total_probes++;
  gcsum = 0;

  // build a probe.
```

FIG. 16D

```
        for(jj = 0; jj < LEN_MER; jj++)
         {
          str[jj] = convert[MM[jj]];
          if(str[jj] == 'c' || str[jj] == 'g')
           gcsum++;
         }
        str[LEN_MER] = '\0';

        // check its GC contents and secondary structure.
        if(gcsum == NUM_GC)
        {
          pass_gc++;

          fprintf(fplog, "pass GCtest: %s\n", str);

          if(!SecondStruct(str))
          {
            strcpy(probe[cnt_prb], str);
            if(++cnt_prb == max_prb)
            {
              // should relocate memory.
              // To simplefy the program, let's just give an error msg.
              fprintf(stderr, "ERROR: Probe array is too small.  cnt_prb is %d\n", cnt_prb);
              exit(1);
            }
          }
          else
          {
            // record the rejected string
            strcpy(sndstr[cnt_snd], str);
            if(++cnt_snd == max_snd)
            {
              fprintf(stderr, "ERROR: Secondary Structure array is too small.  cnt_snd = %d\n",
cnt_snd);
              exit(1);
            }
          }
        }
      }

      fprintf(fp, "\n%d mer probe selection\n", LEN_MER);
      fprintf(fp, "Number of GCs in the probes: %d\n", NUM_GC);
      fprintf(fp, "Score to reject as secondary structure: %d\n",
          SCORE_NEEDED_IN_2ND);
      fprintf(fp, "Score to reject as incompatible: %d\n", CROSSHYB_CUTOFF);
```

FIG. 16E

```
    fprintf(fp, "Compatible test overlap: %d\n", CROSSHYB_OVERLAP);
    fprintf(fp, "Additional equivalent base-pair in compatibility checking: %c%c\n",
        eq1, eq2);
    fprintf(fp, "Simple match cutoff value(including): %d\n", SIMPLE_CUTOFF);
    fprintf(fp, "First N value(including): %d\n", FIRST_N);
    fprintf(fp, "Additional penalty for G or C: %d\n", GC_ADDITION);
    fprintf(fp, "\n\n");

    fprintf(fp, "Total possible %d mers:  %d\n", LEN_MER, total_probes);
    fprintf(fp, "Number passed GC_test :  %d\n", pass_gc);
    fprintf(fp, "Number passed secondary structure test : %d\n", cnt_prb);
//    for(ii = 0; ii < cnt_snd; ii++)
//      fprintf(fp, "%s\n", sndstr[ii]);


    // From the set (call it set1) of probes which passed GC and 2nd structure
    // tests, choose a probe into the final set(set2). Then compare this
    // probe against all the probes left in set1 and throught out the ones
    // that may crosshyb to this probe. From what's left in set1, choose
    // another probe and compary it to the rest of set1...

    compatible = new char [cnt_prb];
    for(ii = 0; ii < cnt_prb; ii++)
    {
      compatible[ii] = 'T';
    }

    // Compatibility check #1: Use weighted scores to penalize neighboring matches.
    //    first_match_score = 1;
    //    if prev pair is a match, current_match_score = prev_match_score*2.
    ii = 0;
    failed_ch = 0;
    while(ii < cnt_prb)
    {
      for(jj = ii+1; jj < cnt_prb; jj++)
      {
       if(compatible[jj] == 'T' &&
         (s=CrossHyb(probe[ii],probe[jj],CROSSHYB_OVERLAP)) >= CROSSHYB_CUTOFF)
         {
          compatible[jj] = 'F';
          failed_ch++;
          fprintf(fplog, "Rejected(%d) %s in slide test for %s\n",
              s, probe[jj], probe[ii]);
         }
      }
```

FIG. 16F

```
    ii++;
    while(ii < cnt_prb && compatible[ii] == 'F')
     ii++;
  }
  fprintf(fp, "Number of probes passed compatibility test: %d\n",
       cnt_prb - failed_ch);

  // Compatibility check #2: Use unweighted score: count unconsecutive matches

  // find the first 'passed' probe.
  ii = 0;
  while(ii < cnt_prb && compatible[ii] == 'F')
   ii++;
  failed_sm = 0;
  while(ii < cnt_prb)
  {
    for(jj = ii+1; jj < cnt_prb; jj++)
    {
      if(compatible[jj] == 'T' &&
        (s=SimpleMatch(probe[ii],probe[jj])) >= SIMPLE_CUTOFF)
      {
        compatible[jj] = 'F';
        fprintf(fplog, "Rejected(%d) %s in simple_match test for %s\n",
             s, probe[jj], probe[ii]);
        failed_sm++;
      }
    }

    ii++;
    while(ii < cnt_prb && compatible[ii] == 'F')
     ii++;
  }
  fprintf(fp, "Number of probes passed simple match test: %d\n",
       cnt_prb - failed_ch - failed_sm);


  // Compatibility check #3: if the first N bases match ANYWHERE in another probe.

  // find the first 'passed' probe.
  ii = 0;
  while(ii < cnt_prb && compatible[ii] == 'F')
   ii++;
  failed_fn = 0;
  while(ii < cnt_prb)
```

FIG. 16G

```
    {
     for(jj = ii+1; jj < cnt_prb; jj++)
      {
       if(compatible[jj] == 'T' &&
          FirstN(probe[ii], probe[jj], FIRST_N) == 'T')
         {
          compatible[jj] = 'F';
          failed_fn++;
          fprintf(fplog, "Rejected %s in FIRSTN test for %s\n",
                probe[jj], probe[ii]);
         }
      }

     ii++;
     while(ii < cnt_prb && compatible[ii] == 'F')
      ii++;
    }

   fprintf(fp, "Number of probes passed FIRSTN compatibility test: %d\n",
        cnt_prb - failed_ch - failed_sm - failed_fn);

   // output.
   jj = 0;
   fprintf(fp, "\nSelected probes are: \n");
   for(ii = 0; ii < cnt_prb; ii++)
   {
     if(compatible[ii] == 'T')
      {
       fprintf(fp, "%s \n", probe[ii]);
       jj++;
      }
   }
}


// Check if 'str' contains a secondary structure.  That is, if there is a
// consecutive 3 bases that matches when 'str' is folded.
// return 1 if found secondary structure, 0 otherwise.

int SecondStruct(const char *str)
{
   int ii, jj, kk, ll;
   int sum, score[32];
   char prev_match;
   char *compl;
```

FIG. 16H

```
char complement[256];
complement['a'] = 't';
complement['t'] = 'a';
complement['c'] = 'g';
complement['g'] = 'c';

ll = strlen(str);
compl = new char [ll+1];
for(ii = 0; ii < ll; ii++)
{
  compl[ii] = complement[str[ii]];
}

for(ii = MATCH_NEEDED_IN_2ND; ii < ll - MATCH_NEEDED_IN_2ND; ii++)
{
  prev_match = 'F';
  sum = 0;
  for(jj = 0; jj < ii; jj++)
  {
    score[jj] = 0;
    kk = ii*2 - jj;
    if(kk < ll)
    {
      if(str[jj] == compl[kk])
      {
        if(prev_match == 'T')
        {
          score[jj] = score[jj-1] * 2;
        }
        else
        {
          score[jj] = 1;
          prev_match = 'T';
        }
      }
      else
      {
        prev_match = 'F';
      }
    }
    sum += score[jj];
  }

  // fprintf(stderr, "2' sum = %d\n", sum);
  if(sum >= SCORE_NEEDED_IN_2ND)
```

FIG. 16I

```
    {
     delete [] compl;
     return 1; // Found a 2nd structure.
    }
}

for(ii = MATCH_NEEDED_IN_2ND - 1; ii < ll - MATCH_NEEDED_IN_2ND; ii++)
{
   prev_match = 'F';
   sum = 0;
   for(jj = 0; jj <= ii; jj++)
   {
      score[jj] = 0;
      kk = ii*2+1 - jj;
      if(kk < ll)
      {
         if(str[jj] == compl[kk])
         {
            if(prev_match == 'T')
            {
               score[jj] = score[jj-1]*2;
            }
            else
            {
               score[jj] = 1;
               prev_match = 'T';
            }
         }
         else
         {
            prev_match = 'F';
         }
      }
      sum += score[jj];
   }

   // fprintf(stderr, "2' sum = %d\n", sum);
   if(sum >= SCORE_NEEDED_IN_2ND)
    {
     delete [] compl;
     return 1; // Found a 2nd structure.
    }
}

delete [] compl;
```

FIG. 16J

```
    return 0;  // No 2nd structure.
}


// check if str1 and str2 can hybridizy together.
// return the max of match scores.
// Assume strlen(str1) == strlen(str2).

int CrossHyb(const char *str1, const char *str2, int overlap)
{
   int ii, jj, len, sum, score, prev_score, max_sum, numGC;
   char prev_match;

   len = strlen(str1);
   max_sum = 0;

   fprintf(fplog, "Sliding test between %s and %s\n", str1, str2);

   for(ii = overlap-len; ii <= len-overlap; ii++)
    {
     numGC = 0;
     sum = 0;
     score = prev_score = 0;
     prev_match = 'F';
     fprintf(fplog, "Compare ");
     for(jj = ii; jj < len && jj - ii < len; jj++)
      {
       if(jj >= 0 && jj - ii >= 0)
        {
         fprintf(fplog, "(%c,%c) ", str1[jj], str2[jj-ii]);
          if((str1[jj] == str2[jj-ii]) ||
            (str1[jj] == eq1 && str2[jj-ii] == eq2) ||
            (str1[jj] == eq2 && str2[jj-ii] == eq1))
           {
             if(((str1[jj]|32) == 'g' && (str2[jj-ii]|32) == 'g') ||
               ((str1[jj]|32) == 'c' && (str2[jj-ii]|32) == 'c'))
               numGC++;

            if(prev_match == 'T')
             {
              score = prev_score*2;
             }
            else
             {
              score = 1;
```

FIG. 16K

```
                prev_match = 'T';
              }
            }
            else
            {
              score = 0;
              prev_match = 'F';
            }
            sum += score;
            prev_score = score;
          }
        }

        fprintf(fplog, "Score=%d\n", sum + numGC*GC_ADDITION);

        if(sum + numGC*GC_ADDITION > max_sum)
          max_sum = sum + numGC*GC_ADDITION;
      }

      fprintf(fplog, "Max score is %d\n", max_sum);
      return max_sum;
}


// Compare 2 strings base to base, 0 to 0, 1 to 1..., no sliding.
// return number of matches.
// Assume strlen(str1) == strlen(str2).

int SimpleMatch(const char *str1, const char *str2)
{
  int ii, sum;

  sum = 0;
  for(ii = 0; ii < strlen(str1); ii++)
  {
    if((str1[ii] == str2[ii]) ||
      (str1[ii] == eq1 && str2[ii] == eq2) ||
      (str1[ii] == eq2 && str2[ii] == eq1))
    {
      sum++;
    }
  }

  return sum;
}
```

FIG. 16L

```
// Check if the first N bases of the two probes are identical.
char FirstN(const char *str1, const char *str2, int N)
{
  int ii;
  char match = 'T';

  if(N > strlen(str1))
   return 'F';

  for(ii = 0; ii < N; ii++)
   {
     if(!((str1[ii] == str2[ii]) ||
       (str1[ii] == eq1 && str2[ii] == eq2) ||
       (str1[ii] == eq2 && str2[ii] == eq1)))
     {
       match = 'F';
       break;
     }
   }

  return match;
}
```

FIG. 16M

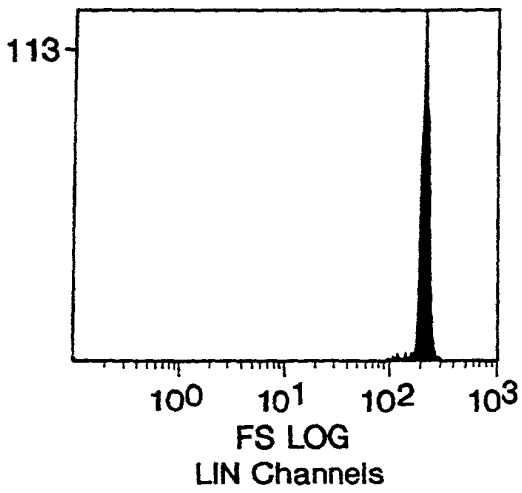


FIG. 17A-1

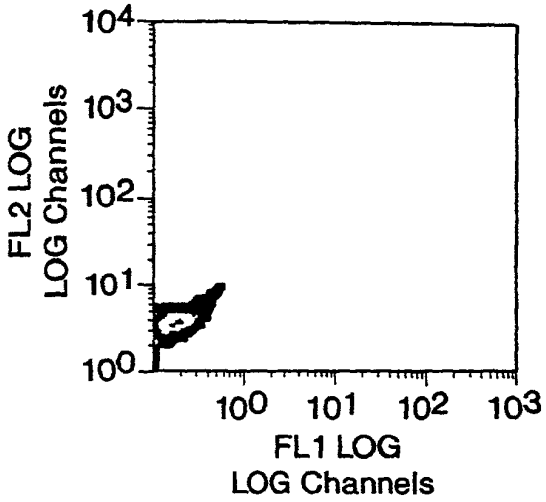


FIG. 17A-2

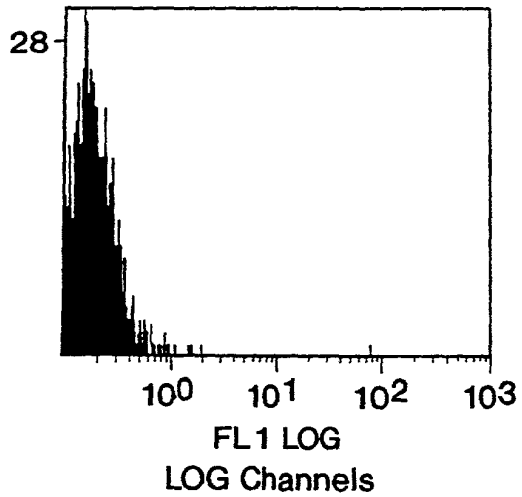


FIG. 17A-3

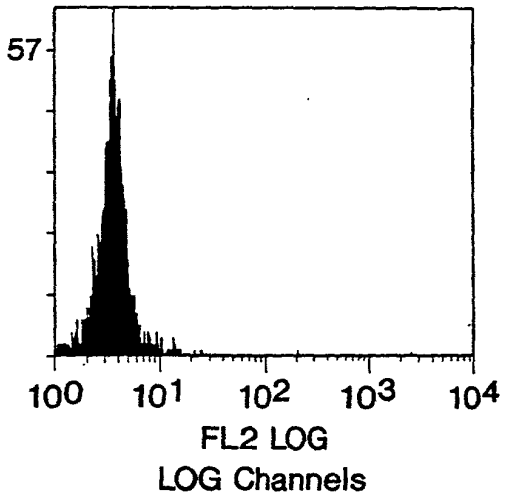


FIG. 17A-4

LIN-LOG Number	%Gated	X-Mean	Units(X axis)
2	0.1	35.9	LOG Channels
2599	86.6	1.8	LOG Channels
2492	83.1	35.5	LOG Channels
238	7.9	661.2	LOG Channels

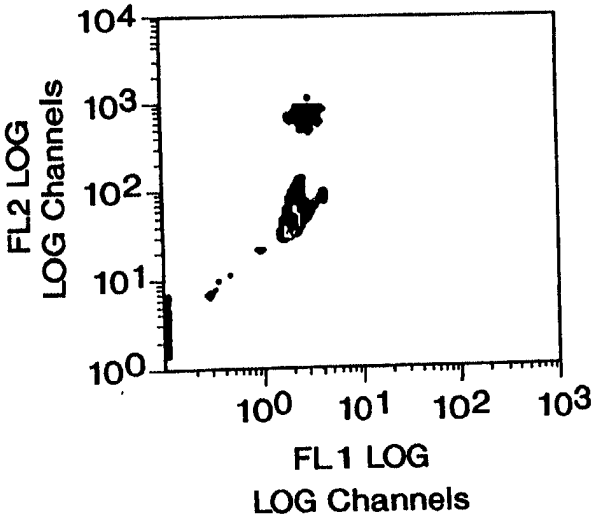


FIG. 17B-1

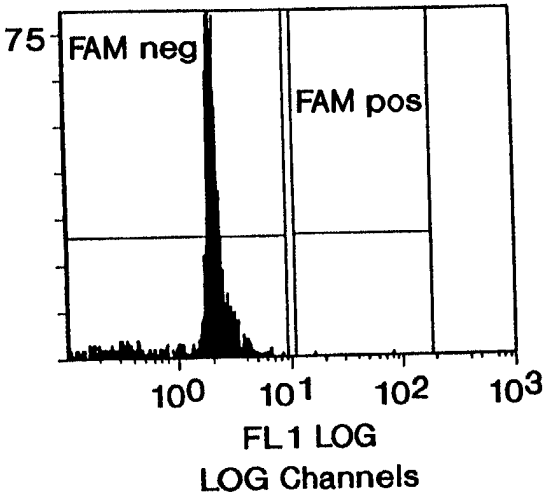


FIG. 17B-2

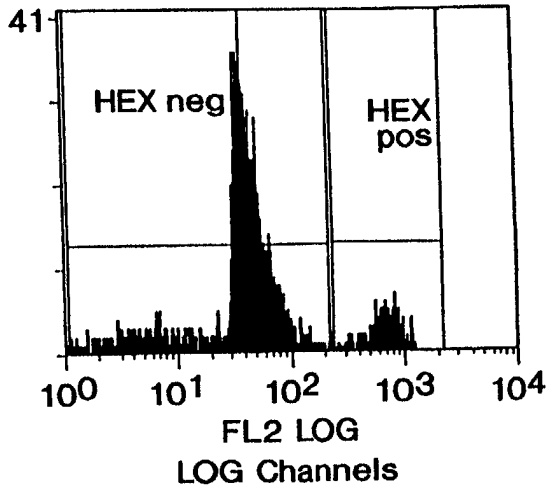
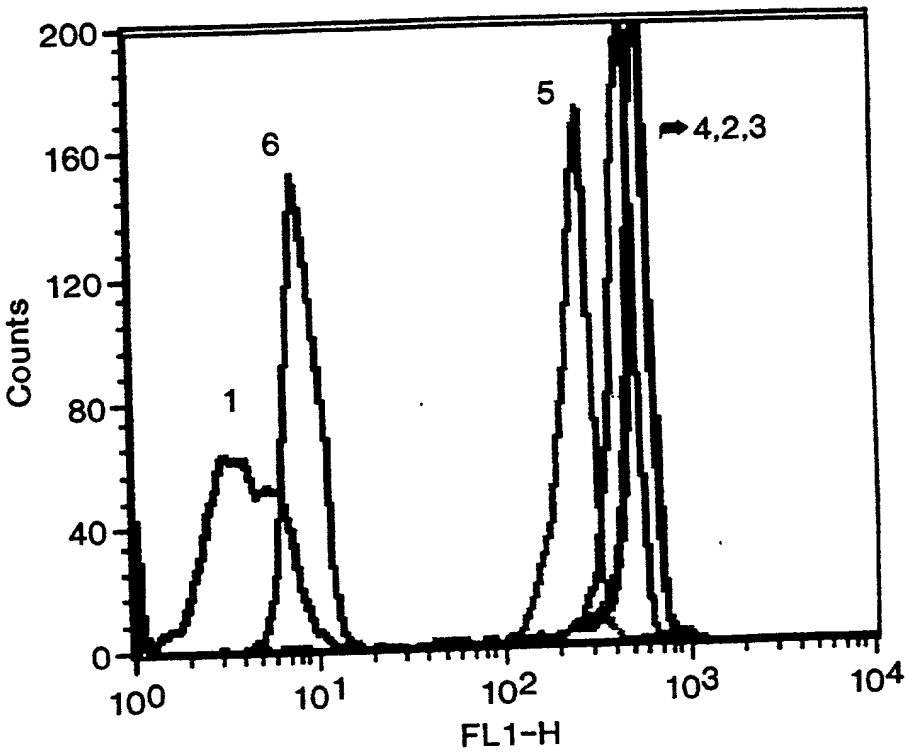
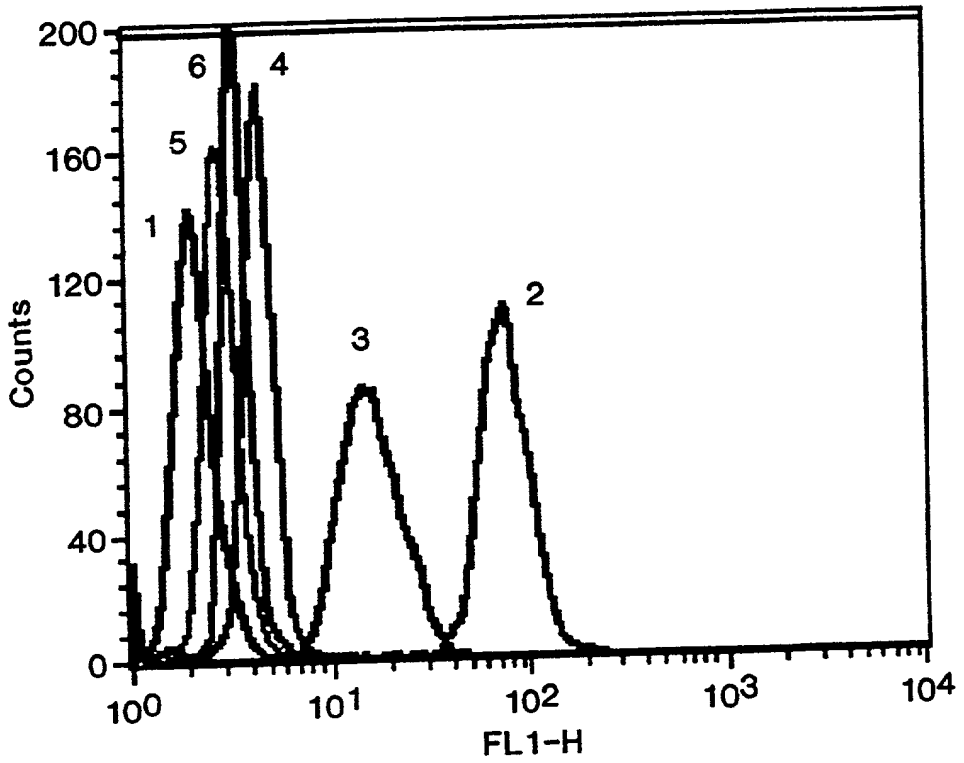


FIG. 17B-3



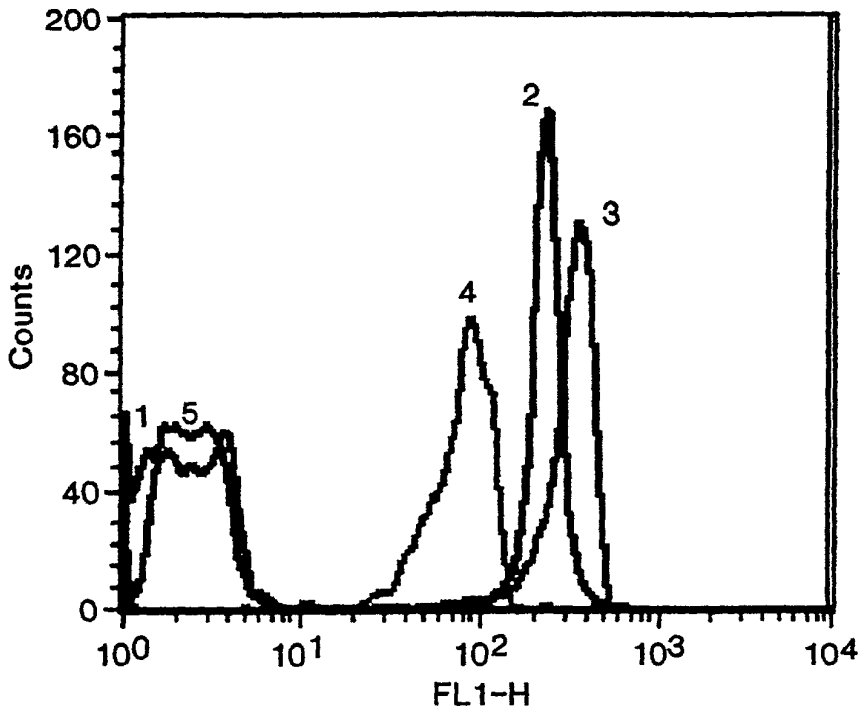
Key	Name
1	5' bead alone
2	5' bead/2 μM 5' c'
3	5' bead/2 μM 60mer DNA
4	5' bead/5 μM 60mer RNA trans.
5	5' bead/1 μM 60mer RNA trans.
6	5' bead/20 μM Non-specific

FIG. 18A



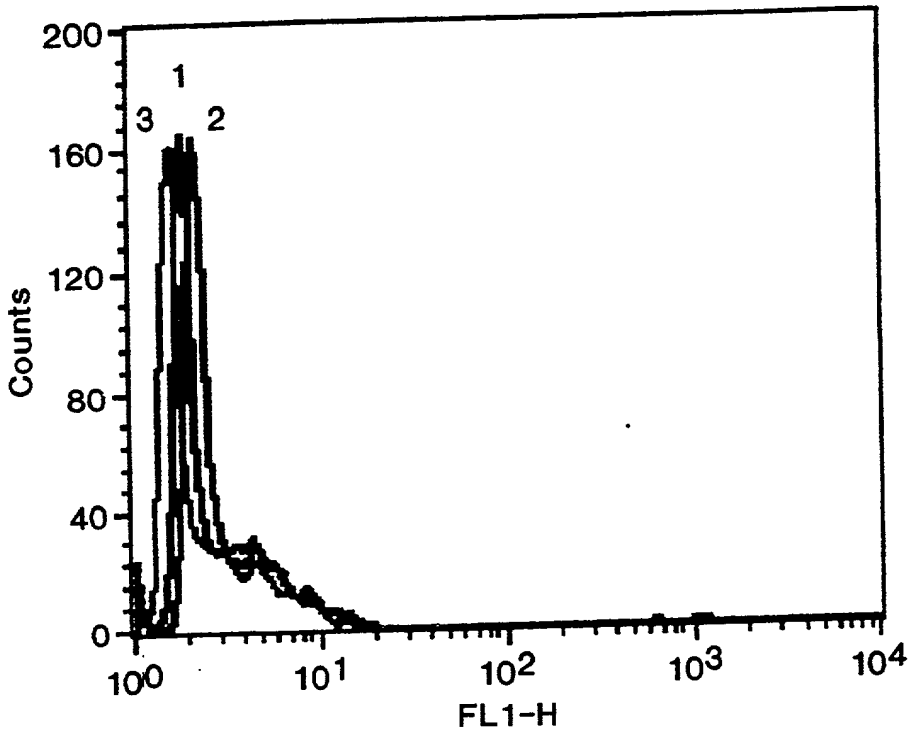
Key	Name
1	3' bead alone
2	3' bead/2 μM 3' c'
3	3' bead/2 μM 60mer DNA
4	3' bead/5 μM 60mer RNA trans.
5	3' bead/1 μM 60mer RNA trans.
6	3' bead/20 μM Non-specific

FIG. 18B



Key	Name
1	Mid bead alone
2	Mid bead/2 μM 60mer DNA
3	Mid bead/5 μM 60mer RNA trans.
4	Mid bead/1 μM 60mer RNA trans.
5	Mid bead/20 μM Non-specific

FIG. 18C



Key	Name
1	NS bead/2 μM 60mer DNA
2	NS bead/5 μM 60mer RNA trans.
3	NS bead/1 μM 60mer RNA trans.

FIG. 18D

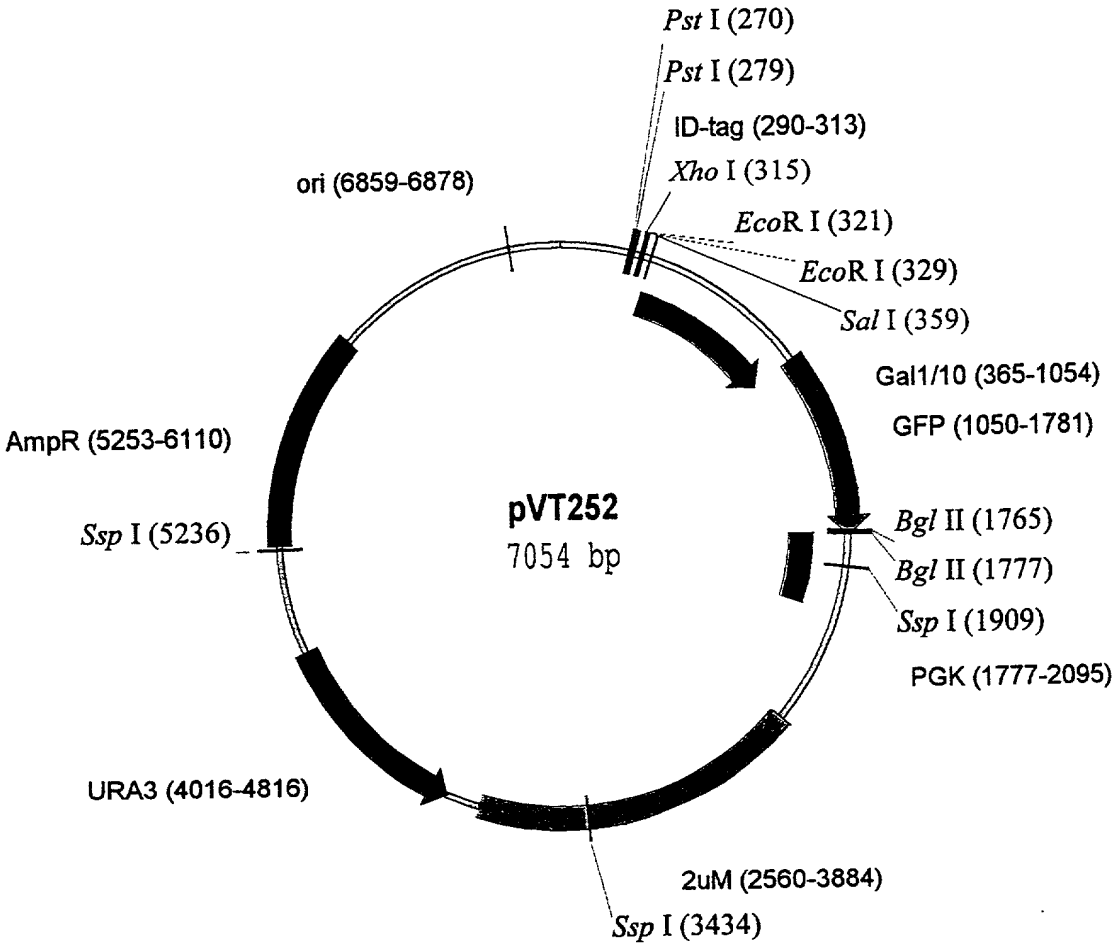

Pst I (270)
Pst I (279)
ID-tag (290-313)
Xho I (315)
EcoR I (321)
EcoR I (329)
Sal I (359)
Gal1/10 (365-1054)
GFP (1050-1781)
Bgl II (1765)
Bgl II (1777)
Ssp I (1909)
PGK (1777-2095)
2uM (2560-3884)
Ssp I (3434)
URA3 (4016-4816)
Ssp I (5236)
AmpR (5253-6110)
ori (6859-6878)
pVT252
7054 bp

FIGURE 19

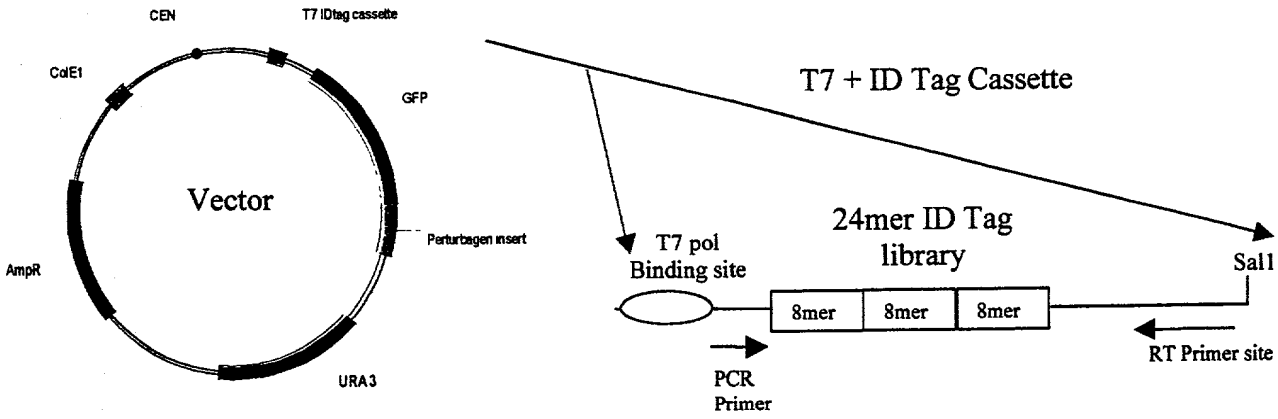
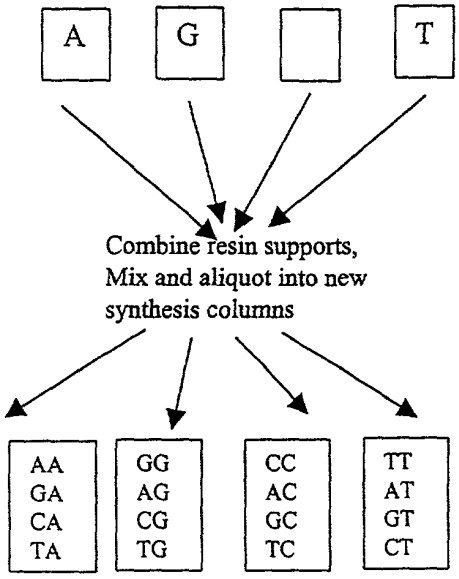

CEN
T7 IDtag cassette
ColE1
GFP
Vector
Perturbagen insert
AmpR
URA3
T7 + ID Tag Cassette
T7 pol
Binding site
24mer ID Tag
library
SalI
8mer
8mer
8mer
RT Primer site
PCR
Primer

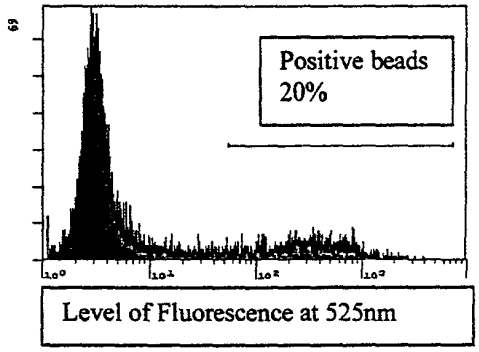
FIGURE 20



Synthesize the nucleotide A on a column and in parallel synthesize G, C and T each on their own oligo synthesis columns.

After second round of synthesis there are a total of 16 di-nucleotide sequences. If columns were combined, mixed, aliquoted, and followed by a third round of synthesis, a total of 64 different tri-nucleotides would be generated.

FIGURE 21



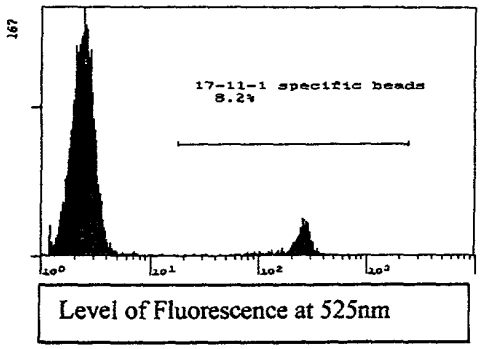
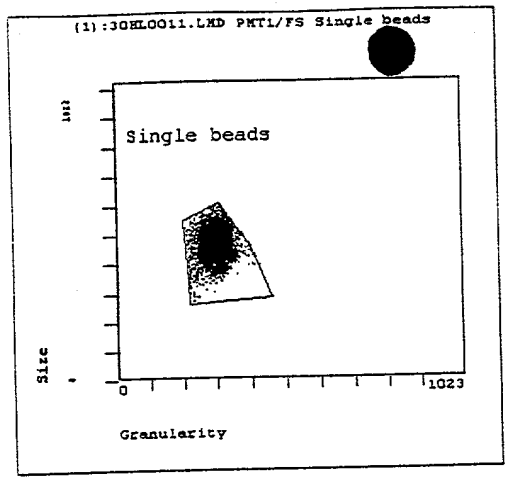


FIGURE 22


(1):3OHL0011.LMD PMT1/FS Single beads
Single beads
Size
0
1023
Granularity

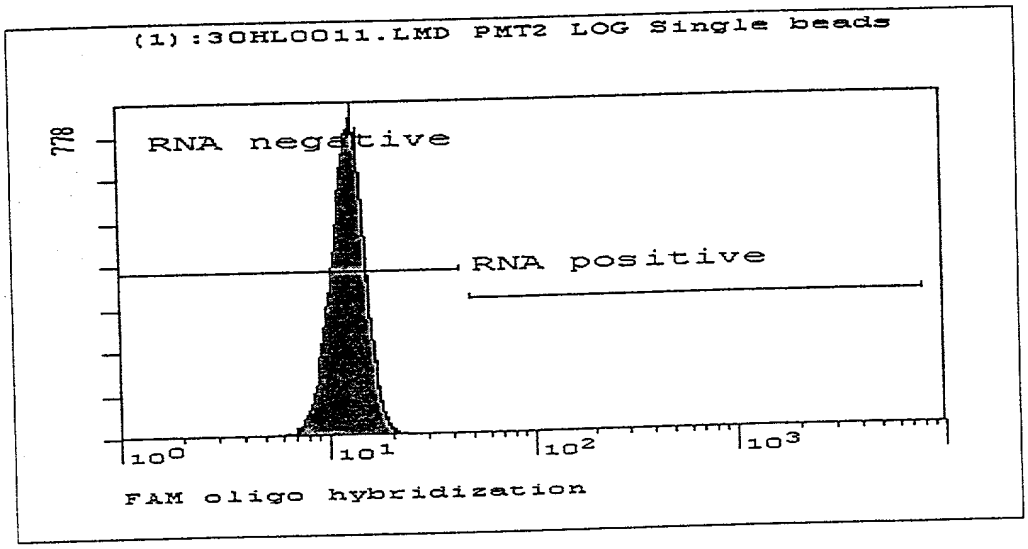
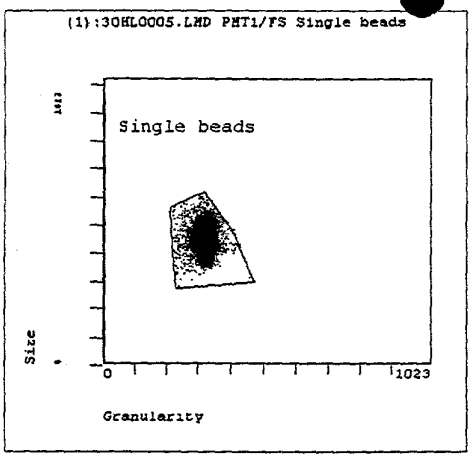

(1):3OHL0011.LMD PMT2 LOG Single beads
778
RNA negative
RNA positive
10^0
10^1
10^2
10^3
FAM oligo hybridization

FIGURE 23


(1):30HL0005.LMD PMT1/FS Single beads
Single beads
Size
0
1023
Granularity

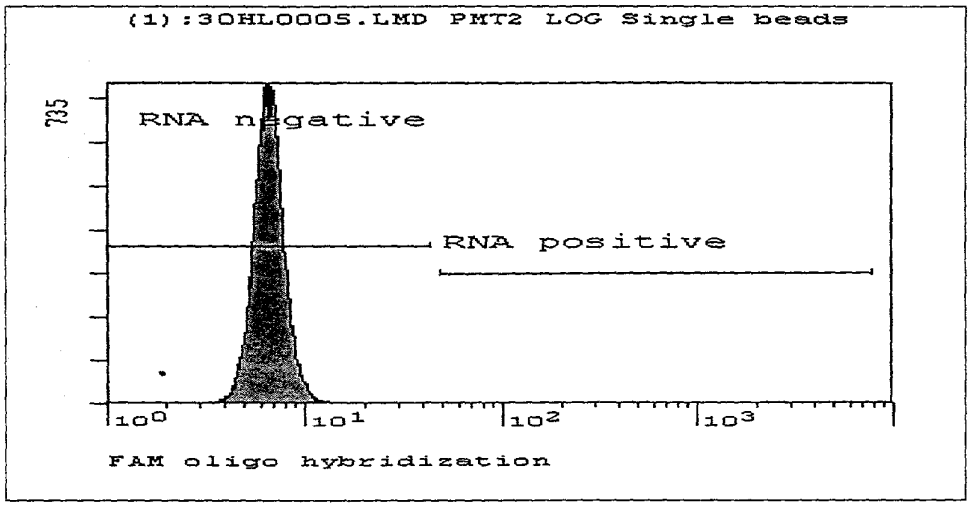
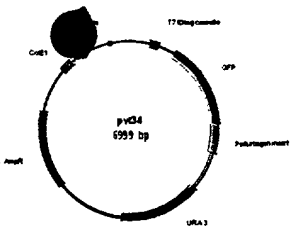

(1):30HL0005.LMD PMT2 LOG Single beads
735
RNA negative
RNA positive
10^0
10^1
10^2
10^3
FAM oligo hybridization

FIGURE 24

FIGURE 25

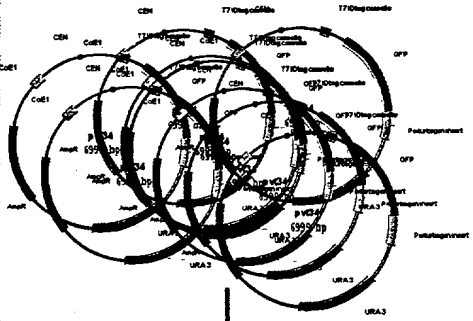


Starting Perturbagen Library in cell grown in dextrose = non expressing conditions

Introduce ID Tagged Perturbagen library into cells and express the perturbagen

Each cell expresses a different perturbagen. Most have no effect... some kill

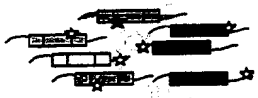
Reisolate plasmid library after passage from induced and non induced grown cells. Keep libraries separate.

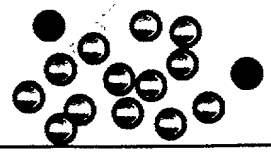


T7 transcribe ID Tags in passaged "Dextrose" library and "Galactose" Library to generate mg quantities of ID Tags as

FITC label Starting Library RNA

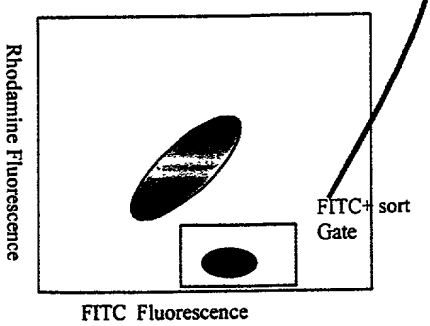
Rhodamine label Passaged Library



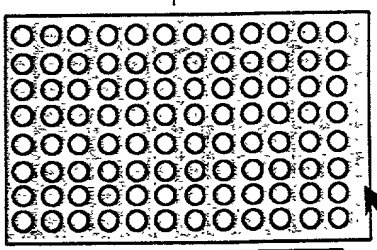


Combine and hybridize RNA from libraries to ID Tag Beads

Sort all Beads on Cell sorter to identify Beads labeled only with FITC+ RNA



Sort single FITC+ beads into 96 well PCR Plate



RT-PCR amplify RNA off beads

Sequence ID Tag and generate complement oligo

Use specific ID-Tag primer and a common vector primer juxtaposed to perturbagen to amplify corresponding perturbagen

Sequence and clone Perturbagen DNA back into expression construct and validate effect